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(57) Abstract

A process for the production of a frozen food product comprising AFP, wherein the conditions are chosen such that the ice-crystals in the product have an aspect ratio of from 1.1 to 1.9.

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FROZEN FOOD WITH ANTIFREEZE PEPTIDES

Technical Field of the Invention

The invention relates to a process for the preparation of a food product containing AFPs and to food products containing AFPs.

Background to the Invention

Anti-freeze peptides (AFPs) have been suggested for improving the freezing tolerance of foodstuffs.

Antifreeze proteins have been described in the literature, see for example Marilyn Griffith and K. Vanya Ewart in Biotechnology Advances, Vol 13, No 3, pp 375-402, 1995. Antifreeze properties generally possess one or more of the following properties: thermal hysteresis, inhibition of ice recrystallisation, control of ice crystal shape and interaction with ice nucleators.

Thermal hysteresis is the best known property of AFPs and the property is normally used to test for the presence of AFPs. Thermal hysteresis results from a lowering of the apparent freezing temperature of a solution containing a thermal hysteresis active AFP without affecting the melting temperature. The identification of sources of AFP by thermal hysteresis tests is widely described in the literature, see for example John G Duman in Cryobiology 30, 322-328 (1993).

Inhibition of ice recrystallisation is another property of AFPs. This activity is also referred to as ice crystal growth suppression. This property can be tested by comparing at a certain point in time the ice crystal size of crystals in the presence of AFP and in the absence of AFP. The application of this method in the testing of fish AFPs is described in US patent 5,118,792 (DNA Plant Technology Corporation)

A third property of AFPs is their ability to influence the shape of ice crystals. This property stems from the selective binding of AFPs to certain faces of the ice crystal and therewith limiting crystal growth in certain directions. The presence of ice crystals having an hexagonal bipyramid shape is then considered indicative of the presence of AFP. This method is for example described for testing the activity of extracellular winter rye AFPs in WO 92/22581 (University of Waterloo).

A fourth property of AFPs is their ability to inhibit the activity of ice nucleating substances. This interaction between and AFP and an ice nucleator may for example result in increased thermal hysteresis. This property is for example tested in WO 96/40973 (University of Notre dame du Lac)

AFPs have been suggested for improving the freezing tolerance of products. Many applications have been suggested in this context.

For example AFPs have been suggested for enhancing the cryopreservation of biological materials (WO 91/12718, Agouron Pharmaceuticals, WO 91/10361, The Regents of the University of California). Also AFPs have been suggested to prevent leakage from liposomes e.g. in cosmetic or pharmaceuticals (see WO 96/20695). A further possible application is to increase the freezing tolerance of plants by including therein (or transgenetically producing therein) an AFP (See J. Cell. Biochem. Suppl. vol. 14e, 1990, page 303 XP002030248, Lee et al, abstract R228). Also fish AFPs have been suggested for use in food products for example in frozen yoghurt or ice cream (US 5,620,732 Pillsbury and WO 96/11586, HSC Research and development limited partnership).

Up till now, however the use of AFPs has not been applied on a commercial scale. Applicants are of the opinion that one of the reasons for the lack of commercial implementation is that

although many AFPs have been described, in practice the implementation in actual commercial products encounters serious problems.

Applicants have found that one of the key reasons for these problems is that out of the great number of AFPs that have been described in the literature only a limited set of AFPs can suitably be applied for each application; also applicants have found that this selection of suitable AFPs is dependent on the desired application and/ or product attributes to be achieved.

WO 90/13571 discloses antifreeze peptides produced chemically or by recombinant DNA techniques. The AFPs can suitably be used in food-products such as ice-cream. Example 3B shows modified ice-crystal shapes if a water-ice mixture is frozen into a film in combination with 0.01 wt% of AFP.

WO 92/22581 discloses AFPs from plants which can be used for controlling ice crystal growth in ice-cream. This document also describes a process for extracting a polypeptide composition from extracellular spaces of plants by infiltrating leaves with an extraction medium without rupturing the plant cells.

WO 94/03617 discloses the production of AFPs from yeast and their possible use in ice-cream. WO 96/11586 describes fish AFPs produced by microbes.

The present invention aims at providing solutions to the above problems. In particular the invention aims at providing frozen food products having a relatively soft although brittle texture, said texture being maintained upon prolonged storage at low temperatures.

Surprisingly it has been found that AFPs can be conveniently incorporated in frozen food products to result in the desired

product properties as long as the processing conditions are varied such that the ice-crystal shape satisfies specific requirements.

Accordingly in a first aspect, the invention relates to a process for the production of a frozen food product comprising AFP, wherein the conditions are chosen such that the ice-crystals in the product have an aspect ratio of from 1.1 to 1.9.

If food products are frozen, ice-crystals are formed throughout the product. If AFPs are included in food products to be frozen this may lead to a change in ice-recrystallisation properties. Aggregation of the ice-crystals of AFP containing products is may cause the brittleness of the product.

Many consumers are in favour of relatively soft and brittle food products or ingredients such as ice-cream and water-ice. For example soft water-ice can be used as an attractive ingredient in frozen confectionery products, also relatively brittle ice-cream is liked by a large group of consumers.

Surprisingly we have found that AFPs offer the opportunity to formulate frozen food products which on the one hand are relatively soft but brittle and on the other hand retain improved ice-recrystallisation properties. Applicants have found that surprisingly this advantageous combination of properties can be achieved if the product contains AFPs and has an aspect ratio of the ice-crystals in the product is between 1.1 and 1.9.

The aspect ratio of ice-crystals is defined as the ratio of the length and the breadth of the ice-crystals. An aspect ratio of between 1.1 and 1.9 corresponds to roundish ice-crystals, which are not elongated in shape. The aspect ratio of crystals can be determined by any suitable method. A

preferred method is illustrated in the examples. Preferably the ratio is between 1.2 and 1.8, most preferred between 1.3 and 1.7.

Preferably the frozen products of the invention are brittle. Preferably the minimum layer thickness at which fraction behaviour can be observed is less than 10 mm, more preferred from 1 to 5 mm. Fracture behaviour can either be measured (by preparing layers of varying thickness and determining at which minimum thickness fracture behaviour occurs)or calculated from the Young's Modulus as described in the examples.

During the formulation and subsequent freezing of food products several parameters can influence the aspect ratio of the ice-crystals to be formed. Examples of factors influencing the aspect ratio are given below. Applicants believe that it is well-within the ability of the skilled person to choose those conditions such that the aspect ratio of the ice-crystals falls within the desired range.

One factor influencing the aspect ratio of ice-crystals is the rate of freezing the product. Generally speaking an increase in the rate of freezing leads to a decrease in the ice crystal aspect ratio. In this context the temperature of freezing may influence the rate of freezing and therewith the aspect ration of the ice crystals. In this context freezing processes including a hardening step e.g. at a temperature below -30 Fahrenheit are sometimes preferred. The storage temperature and storage time may equally influence the aspect ratio, whereby higher storage temperatures and/or longer storage times tend to favour the formation of high aspect ratios.

Another factor influencing the aspect ratio of ice-crystals is the mobility of the product during freezing. For example

if a liquid water-ice or ice-cream mix is to be frozen, quiescently freezing will lead to a fairly high aspect ratio for the ice-crystals, while stirring leads to a lower aspect ratio. High shear mixing will lead to even lower aspect ratios.

Another factor to influence the aspect ratio of the ice crystals is the presence and amounts of ingredients. For example the presence of ingredients which tend to form a network structure in the product (e.g. gums or fats) may lead to a lower aspect ratio than in products without these ingredients. Also other ingredients may lead to lower aspect ratios, for example high solids levels e.g. high sugar levels may lead to low aspect ratios.

Finally the nature and amount of the AFPs present may lead to a change in aspect ratios. Some AFPs seem to favour the formation of low aspect ratios, while other AFPs seem to induce higher aspect ratios. A sutiable test to select these AFPs is described in the examples. Variation in the amount of AFPs may lead to a change in aspect ratios.

According to a second embodiment, the invention relates to a process for the production of a frozen food product comprising AFP, wherein the formulation, freezing and storage conditions are chosen such that the ice-crystals in the product have an aspect ratio of from 1.1 and 1.9.

The process of the invention can be applied to any frozen food product containing AFPs. Examples of suitable products are sauces, meals etc. Preferred food products are frozen confectionery products such as ice-cream and water-ice.

Applicants have found that the AFPs for use in the process of the invention can come from a variety of sources such as plants, fishes, insects and microorganisms. Both natural occurring species may be used or species which have been

obtained through genetic modification. For example micro-organisms or plants may be genetically modified to express AFPs and the AFPs may then be used in accordance to the present invention.

Genetic manipulation techniques may be used to produce AFPs as follows: An appropriate host cell or organism would be transformed by a gene construct that contains the desired polypeptide. The nucleotide sequence coding for the polypeptide can be inserted into a suitable expression vector encoding the necessary elements for transcription and translation and in such a manner that they will be expressed under appropriate conditions (eg in proper orientation and correct reading frame and with appropriate targeting and expression sequences). The methods required to construct these expression vectors are well known to those skilled in the art.

A number of expression systems may be utilised to express the heat stable polypeptide coding sequence. These include, but are not limited to, bacteria, yeast insect cell systems, plant cell culture systems and plants all transformed with the appropriate expression vectors.

A wide variety of plants and plant cell systems can be transformed with the nucleic acid constructs of the desired polypeptides. Preferred embodiments would include, but are not limited to, maize, tomato, tobacco, carrots, strawberries, rape seed and sugar beet.

For the purpose of the invention preferred AFPs are derived from fish. Especially preferred is the use of from plant origin (i.e. proteins directly obtained from planst or these proteins transgenically produced by other organisms), especially derived from winter-rye or perennial grasses.

For some natural sources the AFPs may consist of a mixture of two or more different AFPs.

Preferably those AFPs are chosen which have significant ice-recrystallisation inhibition properties. A suitable test for determining the recrystallisation properties is indicated in the examples. Preferably AFPs in accordance to the invention provide an ice particle size upon recrystallisation - preferably as measured in accordance to the examples- of less than 20 μm, more preferred from 5 to 15 μm. It is believed that the small ice-crystal size combined with the specific aspect ratio is especially advantageous to obtain the desirable structural features.

A very advantageous embodiment of the invention relates to product formulations which are chosen such that in the preparation of the product quiescent freezing conditions can be used, while still obtaining the aspect ratio as defined above.

Examples of such food products are: frozen confectionery mixes such as ice-cream mixes and water-ice mixes which are intended to be stored at ambient or refrigerator temperature. Suitable product forms are for example: a powder mix which is packed for example in a bag or in sachets. Said mix being capable of forming the basis of the frozen food product e.g. after addition of water and optionally other ingredients and -optional- aeration.

Another example of a suitable mix could be a liquid mix (optionally aerated) which, if necessary after addition of further components and optional further aeration can be frozen.

The clear advantage of the above mentioned mixes is that the presence of the AFP ingredient enables the mixes to be frozen under quiescent conditions, for example in a shop or home freezer.

Very conveniently these mixes are packed in closed containers

(e.g. cartons, bags, boxes, plastic containers etc). For single portions the pack size will generally be from 10 to 1000 g. For multiple portions pack sizes of up to 500 kg may be suitable. Generally the pack size will be from 10 g to 5000 g.

As indicated above the preferred products wherein the AFPs are used are frozen confectionery product such as ice-cream or water-ice. Preferably the level of AFPs is from 0.0001 to 0.5 wt% based on the final product. If dry-mixes or concentrates are used, the concentration may be higher in order to ensure that the level in the final frozen product is within the above ranges.

Surprisingly it has been found that compositions of the invention can contain very low amounts of AFPs while still being of good quality.

Up till now the general belief has been that fairly high levels of AFPs are required to obtain a reasonable improvement of recrystallisation properties. The reason for this is that it is commonly believed that the AFPs act on significant parts of the surface of the ice-crystals and therefore need to be present at fairly high levels e.g. 0.01 wt% or more to get a reasonable effect.
Surprisingly it has now also been found that for frozen products improved recrystallisation properties and increased temperature tolerance can already be obtained if low levels of AFPs are used.

Surprisingly it has been found that the level of AFPs can be as low as 0.1 to 50 ppm while still providing adequate recrystallisation properties and temperature tolerance in frozen confectionery products. Although applicants do by no means wish to be bound by any theory, the reason for this may be that the interaction between the solids of the frozen confectionery and the AFPs provides an excellent mechanism

for inhibiting crystal growth. Most conveniently the level of AFP is from 1 to 40 ppm, especially preferred from 2 to 10 ppm.

For the purpose of the invention the term frozen confectionery product includes milk containing frozen confections such as ice-cream, frozen yoghurt, sherbet, sorbet, ice milk and frozen custard, water-ices, granitas and frozen fruit purees. For some applications the use of AFPs in frozen fermented food products is less preferred.

Preferably the level of solids in the frozen confection (e.g. sugar, fat, flavouring etc) is more than 30 wt%, more preferred from 40 to 70wt%.

In a very preferred embodiment of the invention the soft but brittle frozen confectionery formulations are used to create texture contrast in ice confections. Preferably such ice-confections contain as discrete elements in their structure the AFP containing composition in accordance to the invention. For example a relatively hard ice-cream core can be coated with a thin layer of the composition of the invention therewith providing a relatively soft but brittle outer layer surrounding the ice-cream core. Another embodiment could be the incorporation of the formulation of the invention as inclusions in ice-confections. A third embodiment would be the alternating of layers of ice-cream with the formulation of the invention to create thin soft but brittle layers alternating with the ice-cream layers.

Example I

Isolation of AFPs from winter-rye.

Winter rye (Halo variety) was cut in January (mean temperature in that month was 3.5 °C ensuring the appropriate cold acclimatization of the plants). The tissue was rapidly transported into the laboratory for further handling and washed thoroughly with water to remove dirt.

400 g of the clippings were homogenised at ambient temperature in a Waring blender with 800 g water until the leaf tissue was completely disrupted. The AFP rich juice was collected by filtering through 4 layers of muslin.

The AFP rich juice was then subjected to a temperature treatment by boiling the juice for 10 minutes. This caused the precipitation of protein while the AFP for use in accordance to the invention remained in solution. The supernatant was separated from the precipitate by centrifuging at 15,000 g for 20 minutes or by further filtration through muslin.

The AFPs can be isolated from the supernatant by freeze drying.

Example II

Isolation of AFPs from grass.

Mixed grass tissue (containing Poa *trivialis*, Lolium *perenne*, Holcus *lanatus* and Bromus *sterilis*) was cut in January (mean temperature in that month was 3.5 °C ensuring the appropriate cold acclimatization of the plants). The grass tissue was rapidly transported into the laboratory for further handling and washed thoroughly with water to remove dirt.

500 g of grass clippings was placed in a 650 Watt microwave oven and heated at full power for 5 minutes, whereby the temperature was raised to 85 to 100°C. The grass clippings were then cooled to ambient temperature.

After the heating step the AFP rich juice was separated from the clippings by filtering. The mass was stirred continuously for 5 minutes in the presence of an equal volume of water and then squeezed through 3 layers of muslin.

The supernatant can be freeze dried to isolate the AFP.

Example III

A pre-mix for preparing ice-cream was made by mixing:

Ingredient	% by weight
skimmed milk powder	11.39
sucrose	3.14
maltodextrine (MD40)	4.00
corn syrup 63DE	20.71
butteroil	9.00
monoglyceride (palmitate)	0.45
vanillin	0.01
locust bean gum	0.07
guar gum	0.05
carragenan	0.02
microcrystalline cellulose	0.24
gelatin	0.14
AFP (of example I*)	0.01 or none(control)
water	balance

*Note AFP is added as concentrated solution; percentage refers to amount of AFP

This mix can conveniently be stored at ambient temperature e.g. in a plastic container.

The mixture can be whipped with a conventional house-hold mixer to an overrun of about 100%, followed by quiescently freezing into a house-hold freezer.

After two months storage the composition according to the invention had a markedly better texture than the control sample.

Example IV

A liquid premix for the preparation of ice-cream was prepared by mixing:

Ingredient	**% by weight**
Skimmed milk powder	10.00
sucrose	13.00
maltodextrine (MD40)	4.00
locust bean gum	0.14
butteroil	8.00
monoglyceride (palmitate)	0.30
vanillin	0.01
AFP (of example II*)	0.01 or none(control)
water	balance

*Note AFP is added as concentrated solution; percentage referes to amount of AFP

Example V

Ice-creams were prepared by freezing and aerating to 70% overrun the formulation according to example IV:

Samples of both products were equilibrated at -18°C in a Prolan environmental cabinet for approximately 12 hours. Microscopic slides were prepared by smearing a thin layer of ice-cream from the centre of thin glass plates.

Each slide was transferred to a temperature controlled microscopic stage (at -18°C) where images of ice-crystals (about 400 individual ice-crystals) were collected and relayed through a video camera to an image storage and analysis system.

The stored ice crystal images were highlighted manually by drawing around the perimeter which then highlights the whole crystal. Images of the highlighted crystals were then measured using the image analysis software which counts the number of pixels required to complete the longest straight line (length), shortest straight line (breadth), the aspect ratio (length/breadth).

The average aspect ratio for the crystals was calculated.

For the control sample the aspect ratio was 1.45

For the sample containing AFP the aspect ratio was 1.7.

Example VI

The brittleness of the ice-cream of example IV was determined by calculations on the fracture behaviour of the ice-cream. Using a 3-Point bend test the Young's Modulus was measured.

The Young's modulus was measured by preparing strips of ice-cream, equilibrating them for 18 hours in a freezer cabinet and transferring to a temperature cabinet. The strips were placed on a 3-point bend rig as described in Handbook of Plastics Test Methods (2nd Edition), ed R.P. Brown, George Godwin Ltd, 1981. Sample testing was carried out immediately at a deformation speed of 50 mm/min. From the force-deformation curve, the initial slope was measured and used to calculate the Young's modulus according to the following equation:

$$\text{Young's Modulus (Pa)} = \frac{\text{slope} \cdot L}{4 \cdot B \cdot W}$$

where L = beam span (110mm), B = sample width, W = sample height. Usually eight samples were tested to give a mean Young's Modulus value.

Using the calculations described by Williams & Cawood in Polymer Testing 9 15-26 (1990) the fracture toughness can be calculated.

The results were as follows: For the control sample a thickness of 966 m was calculated as being necessary to obtain a brittle layer. For the AFP containing sample brittleness (fracture behaviour) was already found at a thickness of 3 mm. This clearly shows the improved brittleness of products of the invention. Products with AFP were relatively hard.

Example VII

This example describes a methodology to select those AFPs which favour the formation of ice crystal shapes as preferred in the invention.

Ice crystal growth under normal circumstances is along the a-axis of the crystal. If AFPs are present the growth is changed. This selective influencing of the crystal shape can be explained by the fact that AFPs tend to bind to certain parts of the ice crystal and by doing this inhibit the growth of the ice-crystal in certain directions. Binding can for example take place at the prism planes (perpendicular to the a-axis) or at the Pyramidal planes (projecting off these planes).

Applicants have found that AFPs which favour the formation of aspect ratios in accordance to the present invention can be found by selecting those AFPs which tend to bind on the pyramidal plane. The methodology for selecting these specifically binding AFPs can be any suitable methodology. A suitable test uses the so-called "Single ice crystal 'hemisphere' growth experiment, based on the technique described in Knight C.A., C.C. Cheng and A.L. DeVries, Biophys. J. 59 (1991) 409-418, *Adsorption of α-helical antifreeze peptides on specific ice crystal surface planes.*

A well insulated 5l plastic beaker was filled with deionised water, and placed in a temperature-controlled cabinet at -1°C. It was then allowed to freeze slowly from the top. After two days a single crystal of ice approximately 4cm thick covered the beaker. The crystallographic orientation of this crystal was determined using single-crystal X-ray diffraction methods. Cubes of ice, of approximately 2cm dimension, were cut from the large single crystal, such that one surface was parallel to the prism plane, and another parallel to the basal plane. Thus oriented single crystals

of ice were produced.

An apparatus are used consisting of a brass cold finger (approx. 1cm diameter) onto which an oriented seed crystal was frozen. The seed was first hollowed out so that the seed crystal would fit around it. Coolant was then circulated through the finger and the seed became frozen fast to it.

The finger, with the seed crystal, was then dipped into an insulated 100ml beaker containing a solution of the material under investigation. The initial temperature of the solution was room temperature (- 18°C), and the only cooling was provided by the cold finger. Initially the seed crystal partially melted, but it then grew into a single crystal hemisphere. After several hours (6-8) a hemisphere with a diameter of 5-7 cm was formed.

The experiment was carried out with various AFP solutions. The AFP solutions used had a concentration of AFP of 10^{-1} mg/ml.

The hemisphere was then removed from the cold finger, and moved to a temperature controlled cabinet at -15°C. The surface was scraped and it was left in the cabinet at least overnight (16 hours or more). Air was circulated through the cabinet by means of an integral fan. During this time evaporation of the surface layers of the ice occurred. The surface of the ice hemisphere thus came to have a smooth mirror surface. However, for a hemisphere containing AFP rough patches are seen on the surface. These correspond to the patches where the AFP has bound onto the surface of the hemisphere. The large AFP molecules prevent the ice molecules from evaporating, and so a rough mat of AFP molecules is built up on the surface at the surfaces where preferential binding to the ice occurs. As the orientation of the hemisphere is known, and the angular distance between these rough patches and the basal and prism directions can be

measured by means of an optical goniometer the nature of the binding plane can easily be determined.

This test can be used to select those AFPs which tend to bind to the pyramidal planes. For example the AFPs from example I and II tend to bidn to the pyramidal planes. Also a great number of other plant extracts tend to result in pyramidal binding.

It will be well within the ability of the skilled person to use the above test to determine those AFPs which tend to favour the formation of high aspect ratios of ice-crystals. For testing their suitability in frozen products of the invention, the actual product can be made and the aspect ratio of the crystals in the product can be determined.

Example VIII

Test for determining ice crystal size upon recrystallisation.

A sample of an AFP containing solution in water is adjusted to a sucrose level of 30 wt% (If the starting level of the sample is more than 30% this was done by dilution, if the starting level was lower sucrose was added to the 30% level).

Generally the test can be applied to any suitable composition comprising AFP and water. Generally the level of AFP in such a test composition is not very critical and can for example be from 0.0001 to 0.5 wt%, more preferred 0.0005 to 0.1 wt%, most preferred 0.001 to 0.05 wt%, for example 0.01 wt%

A 3 μL drop of the sample is placed on a 22 mm coverslip. A 16 mm diameter cover-slip is then placed on top and a 200 g weight is placed on the sample to ensure a uniform slide thickness. The edges of the coverslip are sealed with clear nail varnish.

The slide is placed on a Linkham THM 600 temperature controlled microscope stage. The stage is cooled rapidly (50 °C per minute) to -40°C to produce a large population of small crystals. The stage temperature is then raised rapidly (50°C per minute) to -6 °C and held at this temperature.

The ice-phase is observed at -6 °C using a Leica Aristoplan microscope. Polarised light conditions in conjunction with a lambda plate were used to enhance the contrast of the ice-crystals. The state of the ice phase (size of ice-crystals) is recorded by 35 mm photomicrography at T=0 and T=1 hour. Whereby an average particle size (visual determination, number average) of below 20 μm, more prefered between 5 and 15 μm indicates preferred AFPs for use in products according to the invention.

Claims

1. A process for the production of a frozen food product comprising AFP, wherein the conditions are chosen such that the ice-crystals in the product have an aspect ratio of from 1.1 to 1.9.

2. A process according to claim 1 wherein the conditions for influencing the aspect ratio are selected from the group of: rate of freezing, mobility of product during freezing, storage temperature and time, formulation of the product and nature and amount of AFPs and combinations thereof.

3. A process according to claim 1, wherein the frozen food product is a frozen confectionery product.

4. A frozen confectionery product comprising from 0.0001 to 0.5 wt% of AFPs, said product having an ice-crystal aspect ratio of from 1.1 to 1.9.

5. Frozen confectionery product according to claim 4, wherein the AFPs preferentially bind to the pyramidal planes of ice crystals.

6. Frozen confectionery product having a texture contrast, said product comprising discrete elements of a confectionery product of claim 4.

7. Frozen confectionery product according to claim 6, comprising thin ice-cream layers alternating with thin water-ice layers, wherein the water-ice layers comprise from 0.0001 to 0.5 wt% of AFPs and have an ice-crystal aspect ratio of from 1.9 to 3.0.

8. Ice-cream mix suitable for use in the preparation of a

frozen confectionery product of claim 4.

9. Ice-cream mix according to claim 8, wherein the preparation involves aeration and quiescent freezing.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
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B. FIELDS SEARCHED

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IPC 6 A23G

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92 22581 A (M. GRIFFITH) 23 December 1992 cited in the application see page 30, line 20-30	1-4,6
Y		5
Y	see page 21, line 11 - page 22, line 17; figures 6,8	8,9

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
19 November 1997	04/12/1997

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Guyon, R

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INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/EP 97/03636

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	P.J. LILLFORD ET AL.: "Antifreeze proteins" JOURNAL OF FOOD ENGINEERING, vol. 22, 1994, GREAT BRITAIN, pages 475-482, XP002047525 see page 480, line 30 - line 38 ---	1,5
A	PATENT ABSTRACTS OF JAPAN vol. 18, no. 537 (C-1260), 13 October 1994 & JP 06 189686 A (SATO NORIO ET AL.), 12 July 1994, see abstract ---	1
A	US 3 897 571 A (B. HOMLER ET AL.) 29 July 1975 see column 1, line 40-57 see column 2, line 45 - column 3, line 36 ---	1
Y	R. E. FEENEY ET AL.: "ANTIFREEZE PROTEINS" FOOD TECHNOLOGY, vol. 47, no. 1, January 1993, CHICAGO, pages 82-90, XP002022179 see page 84, column 2 - page 85, column 1; figures 4,5 see page 87, column 1 - column 2 ---	1-4,6,8, 9
Y	W. S. ARBUCKLE: "ICE CREAM" 1987 , THE AVI PUBLISHING COMPANY , 4TH. EDITION 1987 XP002022180 see page 232, line 1 - page 238 see figures 12.1,12.4 ---	1-4,6,8, 9
A	WO 96 11586 A (G. FLETCHER ET AL.) 25 April 1996 cited in the application ---	
A	WO 96 16557 A (COX D. R. G. ET AL) 6 June 1996 see page 4, line 12 - page 5, line 15 see page 6, line 25 - page 8, line 13 ---	1,9
A	US 4 500 553 A (L. G. LIGGETT ET AL.) 19 February 1985 see column 2, line 59-63 see column 3, line 41-54 ---	1
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